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In the third year of this grant, we are about to complete one experiment, and have initiated another. In Experiment 1, we assessed whether lentiviral delivery of the antiapoptotic gene Bcl-2 could prevent the functional and structural consequences of MPTP toxicity in nonhuman primates. Our preliminary data indicate that lentiviral delivery of Bcl2 did not influence MPTP-mediated motor deficits as measured on a clinical rating scale and an operant hand reach task, likely because Bcl-2 may not be able to preserve striatal dopaminergic innervation. We are currently evaluating Neurochemically and morphologically whether lenti-Bcl2 provided neuroprotection at the level of the substantia nigra. We have just initiated a second experiment in which aged monkeys are assessed for levodopa-induced dyskinesias and given a fluorodopa PET scan. They then receive lenti-GDNF under the control of the tetracycline promoter. Three months following lenti-GDNF, they are again assessed for levodopa-induced dyskinesias and given a fluorodopa PET scan. Then half of the aged monkeys receive tetracycline to shut of the GDNF expression in vivo. Monkeys are assessed for levodopa-induced dyskinesias and given a fluorodopa PET scan 3 and 6 months later. This study will determine whether we can control gene expression in vivo and make gene therapy safe for clinical use.

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#### **4) Introduction**

Parkinson's disease (PD) is a chronic neurodegenerative disorder that affects over 1,000,000 Americans. Symptoms include tremor, bradykinesia and rigidity, all of which invariably increase in severity as the disease progresses. Pathologically, there is progressive loss of striatal dopamine and degeneration of dopaminergic neurons within the substantia nigra pars compacta. Palliative symptomatic treatment can be achieved by dopamine (DA) replacement therapy using the dopamine precursor, levodopa. However, "wearing off effects" with disabling dyskinesias complicates symptomatic treatments. As PD progresses, motor and nonmotor symptoms emerge which are not responsive to levodopa. Since treated patients show a life expectancy similar to age-matched controls, patients can survive with crippling symptoms for many years. Thus, new innovative treatment strategies are needed to sustain the quality of life for these individuals. Recently, surgical treatment strategies such as neural transplantation (e.g. 1), pallidotomy (e.g. 2) or deep brain stimulation (e.g. 3) have gained considerable attention for the treatment of PD. However, preventing neuronal degeneration, rather than replacing neurons or disrupting basal ganglia circuitry may be a more parsimonious way of sustaining nigrostriatal and clinical function in patients with PD. The present proposal plans to use a neuroprotection strategy and determine whether delivery of the antiapoptotic protein BCL-2 via *in vivo* gene therapy systems can reverse motor deficits and nigrostriatal dysfunction in MPTP-treated nonhuman primates. Additionally, we have just begun a new study in aged nonhuman primates that serve as a follow-up to our recent publication in Science demonstrating the ability of lentiviral delivery of the trophic factor GDNF to prevent degeneration and enhance nigrostriatal function in nonhuman primate models of Parkinson's disease. In this new study, we will determine whether we can control transgene expression using the tet-off system.

#### **5) Body of Report**

The studies being performed are using lentivirus as an *in vivo* delivery system for BCL-2. We previously demonstrated that this delivery system is effective in delivering therapeutic proteins in nonhuman primate models of Parkinson's disease

**Lentiviral-delivered Bcl-2:** This experiment examines the effects of lenti-Bcl-2 upon motor behavior and nigrostriatal degeneration in MPTP-treated monkeys. Initially, twenty young adult Rhesus were initially trained 3 days per week until asymptotic performance was achieved on a hand-reach task in which the time to pick up food treats out of recessed wells was measured. Each experimental day, monkeys received 10 trials per hand. Once per week, monkeys were also evaluated on a modified parkinsonian clinical rating scale (CRS). All monkeys then received an injection of 3mg MPTP-HCl into the right carotid artery, initiating a parkinsonian state. One week later, monkeys were evaluated on the CRS. Only monkeys displaying severe hemiparkinsonism with the classic crooked arm posture and dragging leg on the left side continued in the study (n=10). It is our experience that monkeys with this behavioral phenotype display the most severe lesions neuroanatomically and do not display spontaneous recovery behaviorally. Based upon CRS scores, monkeys were matched into two groups of five monkeys which received that day lenti- $\beta$ Gal or lenti-Bcl-2 treatment. Using MRI guidance, all monkeys received lentivirus injections into the substantia nigra (5 $\mu$ l) on the right side.. One week later, monkeys began retesting on the hand reach task 3 times per week for 3 weeks per month. For statistical analyses, the times for an individual week were combined into a single score. During the weeks of hand reach testing, monkeys were also scored once per week on the CRS. Individuals blinded to the experimental treatment performed all behavioral assessments. Three months after lentivirus treatment, monkeys received a FD PET scan, were sacrificed 24-48h later, and histologically processing.

Prior to MPTP treatment, all young adult monkeys scored zero on the CRS. Following MPTP, All monkeys displayed scores on the clinical rating scale between 9-12. Following lentivirus treatment, there were no significant differences in CRS scores were seen between the two groups (Kolmogorov-Smirnov test;  $p>0.05$ ).

Lenti-Bcl2 treated animals also failed to improve performance on the operant hand reach task. Under-pre-MPTP conditions, animals in both groups performed this task with similar speed. For the “unaffected” right hand, no differences in motor function were discerned for either group relative to pre-MPTP levels or to each other ( $p>0.05$ ). Following MPTP, all lenti- $\beta$ Gal and lenti-Bcl2-treated animals were severely impaired, with monkeys often not performing at all, or requiring more than the maximally-allowed 30 seconds.

Just prior to sacrifice, all monkeys underwent FD PET scans. These PET scans are still being evaluated and the Ki values quantified. As of the writing of this report, the brains from these animals are being sectioned and the punches for neurochemistry are being processed.

**Control of transgene expression in nonhuman primate models of PD:** We previously demonstrated that lentivirally-delivered GDNF provides potent function, neuroanatomical, and neurochemical effects on the degenerating nonhuman primate nigrostriatal system (see previous progress reports and Kordower et al., 2000). In fact, lenti-GDNF was so potent that some parameters displayed hyperdopaminergic function. Prior to going to clinical trials, it is essential that we have control of the GDNF expression in vivo. In this regard, we have designed the following experiment. Ten aged Rhesus monkeys comprise this experiment. They are evaluated for baseline levels of levodopa-induced dyskinesias (done) and receive a fluorodopa PET scan (done). Then they all will receive a unilateral intrastriatal injections of lenti-GDNF under the control of the tetracycline promoter. Three months later they will be evaluated for levodopa-induced dyskinesias, testing the hypothesis that lenti-GDNF mediated increases in the number of terminals will move the dyskinesia dose response profile to the left (fewer dyskinesias) and determine empirically that increasing dopaminergic tone in this was does not increase the expression of dyskinesias. These monkeys will also get a second fluorodopa PET scan. Based upon our previous data (Kordower et al., 2000), all aged monkeys should display a robust increase in fluorodopa uptake. Then half of the animals will receive chronic tetracycline in the drinking water, shutting off the GDNF gene. Both 3 and 6 months

later, the monkeys will again be tested for levodopa-induced dyskinesias and receive PET scans. We hypothesize that the “tet” exposed animals will reverse the expression of levodopa-induced dyskinesias and reverse the increase in fluorodopa on PET. These experiments will establish the controllability of gene expression making this procedure safe for clinical trials. This experiment will continue through the third year of funding.

#### **6) Key Research Accomplishments**

Determining whether lentiviral delivery of the apoptotic gene can prevent the functional and structural consequences of the parkinsonian agent MPTP in nonhuman primates.

Establishing whether we can control gene expression of GDNF in aged nonhuman primates.

#### **7) Reportable Outcomes**

none but there will be published reports following the analysis of the above data.

**8) Conclusions:** We cannot at present make any definitive conclusions as we are completing Experiment 1 and starting Experiment 2.

#### **9) References:**

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**11: Appendices:** None